Inspired by Nature: Hydrogels as Versatile Tools for Vascular Engineering

Blache, Ulrich ; Ehrbar, Martin

Abstract: Significance: Diseases related to vascular malfunction, hyper-vascularization, or lack of vascularization are among the leading causes of morbidity and mortality. Engineered, vascularized tissues as well as angiogenic growth factor-releasing hydrogels could replace defective tissues. Further, treatments and testing of novel vascular therapeutics will benefit significantly from models that allow for the study of vascularized tissues under physiological relevant in vitro conditions. Recent Advances: Inspired by fibrin, the provisional matrix during wound healing, naturally derived and synthetic hydrogel scaffolds have been developed for vascular engineering. Today, engineers and biologists use commercially available hydrogels to pre-vascularize tissues, to control the delivery of angiogenic growth factors, and to establish vascular diseases models. Critical Issue: For clinical translation, pre-vascularized tissue constructs must be sufficiently large and stable to substitute function-relevant tissue defects and integrate with host vascular perfusion. Moreover, the continuous integration of knowhow from basic vascular biology with innovative, tailorable materials and advanced manufacturing technologies is key to achieving near-physiological tissue models and new treatments to control vascularization. Future Directions: For transplantation, engineered tissues must comprise hierarchically organized vascular trees of different caliber and function. The development of novel vascularization-promoting or -inhibiting therapeutics will benefit from physiologically relevant vessel models. In addition, tissue models representing treatment-relevant vascular tissue functions will increase the capacity to screen for therapeutic compounds and will significantly reduce the need for animals for their validation.

DOI: https://doi.org/10.1089/wound.2017.0760

Article type: Comprehensive Invited Review

Title: Inspired by Nature: Hydrogels as versatile Tools for Vascular Engineering

*Authors: Ulrich Blache [1, 2], Martin Ehrbar [1]**

[1] Department of Obstetrics, University and University Hospital Zurich, Schmelzbergstrasse 12, 8091 Zurich, Switzerland

[2] Department of Health Sciences and Technology, Institute for Biomechanics, ETH Zurich, 8008 Zurich, Switzerland

Abbreviated title: Hydrogel-based Vascular Engineering

* Corresponding author:

Martin Ehrbar

Schmelzbergstrasse 12

PATG G48

8091 Zürich

Telephone +41442558513

Fax +41 44 255 44 30

Martin.Ehrbar@usz.ch

Word count: 5935

Key words: hydrogel, blood vessel, tissue model, engineering, angiogenesis, growth factor

Abstract:

Significance: Diseases related to vascular malfunction, hyper-vascularization, or lack of vascularization are among the leading causes of morbidity and mortality. Engineered, vascularized tissues as well as angiogenic growth factor releasing hydrogels could replace defective tissues. Furthermore, treatments and testing of novel vascular therapeutics will benefit significantly from models that allow for the study of vascularized tissues under physiological relevant in vitro conditions.

Recent Advances: Inspired by fibrin, the provisional matrix during wound healing, naturally-derived and synthetic hydrogel scaffolds have been developed for vascular engineering. Today, engineers and biologists use commercially available hydrogels to pre-vascularize tissues, to control the delivery of angiogenic growth factors, and to establish vascular diseases models.

Critical Issue: For clinical translation, pre-vascularized tissue constructs must be sufficiently large and stable to substitute function-relevant tissue defects and integrate with host vascular perfusion. Moreover, the continuous integration of knowhow from basic vascular biology with innovative, tailorable materials and advanced manufacturing technologies is key to achieving near-physiological tissue models and new treatments to control vascularization.

Future Directions: For transplantation, engineered tissues must comprise hierarchically organized vascular trees of different caliber and function. The development of novel vascularization promoting or inhibiting therapeutics will benefit from physiologically relevant vessel models. Additionally, tissue models representing treatment-relevant vascular tissue functions will increase the capacity to screen for therapeutic compounds and will significantly reduce the need for animals for their validation.

Table of contents

1.0 Scope and Significance: Diseases related to vascular malfunctions are among the leading causes of morbidity and mortality in the industrialized world. While patients suffer from insufficient vascular perfusion of the heart, the brain, or even peripheral tissues, excessive vascular growth contributes to the pathogenesis of cancer, psoriasis, or diabetic retinopathy. Even though treatments towards insufficient perfusion by growth factor-mediated induction of angiogenesis have delivered promising results in basic research, clinical trials have not met expectations. Additionally, anti-angiogenic approaches towards the reduction of vascularization in most cancers have only resulted in the minimal extension of progression free survival. Obviously, relevant in vitro models to study blood vessel biology and treatment approaches under near-physiological conditions are needed. The engineering of vascularized tissue constructs for in vivo transplantation as well as for modeling diseases in vitro requires appropriate scaffold materials that support cell-specific functions and are compatible with innovative (micro)-manufacturing techniques. Extracellular matrix (ECM)-inspired hydrogels fulfill these requirements and are extremely versatile scaffold materials for vascular engineering**.** Importantly, essential to the success of vascular engineering is the continuous integration of hydrogel innovations with advances in vascular biology and manufacturing techniques. In this review, we discuss hydrogels in vascular engineering, including their in vivo application. We specifically focus on the advancements related to materials research and on the development of vascularized three-dimensional (3D) tissue models.

2.0 Translational Relevance: The continuous integration of novel biomaterials and technologies with vascular biology and medicine is important for the success of vascular engineering. Furthermore, vascularized tissue models are based on hydrogel materials compatible with innovative 3D manufacturing techniques. Vascularized 3D models that resemble the complexity of differentiated human tissues will build an important base to study basic vascular biology, vascularized tissues, and novel therapeutics under fully controlled near-physiological conditions.

3.0 Clinical Relevance: Clinical treatments to improve or inhibit vascularization using drug releasing materials were only partially successful and require further biological understanding and the improvement of current treatment concepts. Engineered, vascularized tissue units that are functional in vivo could be transplanted to treat ischemic tissues. Small, physiologically relevant 3D models of various tissues or cancers will enable the screening and testing of novel therapeutics under defined conditions and will provide a platform for personalized medicine approaches. Furthermore, novel 3D models could help to understand the progression of vascularization-related disease on the cellular level.

4.0 Background/Overview

4.1 Mechanisms of blood vessel development, maturation, and remodeling

The cardiovascular system supplies almost all tissues with gas, nutrients, signaling molecules, and cells. Neovascularization, the formation of new blood vessels, is a fundamental part of tissue formation and repair involving two distinct morphogenetic processes: vasculogenesis and angiogenesis (Fig. 1A). Vasculogenesis denotes the de novo formation of blood vessels from mesodermal-derived precursor cells called angioblasts. The differentiation of angioblasts into endothelial cells and their subsequent assembly into a new primitive blood vessel network mainly occurs during embryonic development. Initially described to take place in the yolk sac of the developing mouse embryo, it became clear that blood vessel initiation by vasculogenesis independently occurs at both intra-embryonic and extra-embryonic sites of the embryo.[1] Interestingly, circulatory endothelial progenitor cells have been shown to enable vasculogenesis in the adult organism where they could play a significant role in tissue repair and regeneration.[2-4] Although relatively limited information on the molecular mechanism of vasculogenesis is available, genetic ablation studies showed that fibroblast growth factor (FGF)-2, bone morphogenetic protein (BMP)-4, and vascular endothelial growth factor (VEGF) are centrally involved in the process of vasculogenesis.[5] In contrast, angiogenesis is the formation of new

blood vessels from preexisting ones and occurs throughout the whole lifespan. Sprouting angiogenesis describes the branching of endothelial cells from existing blood vessels due to the local environment's provision of angiogenic signals.[6-8] Intussusceptive angiogenesis occurs from the formation of pillars in the inside of preexisting capillaries resulting in their longitudinal division "within itself."[9] However, molecular mechanisms leading to intussusceptive angiogenesis remain largely obscure.

Under homeostatic conditions small-caliber blood vessels are composed of an endothelial cell tube and perivascular cells. In this constellation, endothelial cells are quiescent and form stable interactions with each other and with a specialized ECM layer, the basement membrane.[10] Upon metabolic demand or hypoxic conditions pro-angiogenic molecules, such as VEGF, FGF, platelet derived growth factor (PDGF)-BB, stromal cell-derived factor (SDF1)-α, and Angiopoietin (ANG)-2 become available in the local environment and cause the proteolytic remodeling of the vessel basement membrane, the destabilization of endothelial cell-cell contacts, and the extravasation of blood plasma proteins that contribute to the formation of a provisional ECM.[11-13] Interestingly, inflammatory cells upon injury establish quite similar angiogenic growth factor milieus. Subsequently, some endothelial cells via VEGF receptor (VEGFR)-2 and VEGFR-3 mediated signaling acquire a tip cells phenotype, become motile, and form filopodia.[14, 15] Tip cells upregulate the delta-like ligand (DLL)-4 that binds to the Notch receptor of neighboring endothelial cells where it suppresses the expression of VEGFR-2 and VEGFR-3 and promotes the expression of VEGFR-1.[15] As a consequence of the changed VEGFR expression, these cells become less responsive to VEGF-signals and exhibit a stalk cell phenotype showing stronger proliferation and the initiation of lumen formation. To form new vascular networks two tip cells undergo anastomosis, which is initiated by filopodia contacts and results in the fusion of different endothelial sprouts.

Primitive endothelial tube maturation into mature vessels is initiated by the recruitment of perivascular cells that ultimately enwrap the endothelial tubes. Perivascular cells are of

mesenchymal origin and classified as pericytes or vascular smooth muscle cells depending on their localization to small or large vessels, respectively.[16, 17] Among the factors known to play an important role in the recruitment, proliferation, and differentiation of perivascular cells are PDGF-BB, ANG-1, and transforming growth factor (TGF)-β. Due to their stabilizing and regulating role, perivascular cells are generally considered as vascular support cells. Additionally, debate continues over whether perivascular cells are mesenchymal tissue progenitor cells since these cells also reside in the perivascular microenvironment of blood vessels.[18-24]

Angiogenesis is a partially overshooting process and endothelial cells become calmed by perivascular cells once in contact. However, overproduced or redundant vessels need to be pruned. The main cause of vessel regression is interrupted blood flow.[25-27] Mechanistically, upon lumen collapse vessel regression is achieved by increased apoptosis or migration of endothelial cells.[27-30] However, and conversely, the lumen collapse during vessels regression has also been shown to be the consequence of endothelial cell apoptosis.[31] Furthermore, the finding that perivascular cells are actively involved in vessel pruning supports the idea that vessel regression is not a passive process.[32] Remarkably, vessel formation and vessel pruning can occur simultaneously within the same vascular network.[33]

4.2 Extracellular matrix as template for neovascularization during tissue repair

Neovascularization is crucial to tissue development and homeostasis. Hence, neovascularization is also key to wound healing and tissue repair. The pivotal step of blood vessel establishment during wound healing is the recruitment of participating cells into the defective tissue. This cell recruitment requires a supportive matrix, which is established during the initial steps of wound healing by the release of fibrinogen, plasma fibronectin, and platelets from leaky blood vessels. The resulting fibronectin and platelet enriched fibrin clot acts as a temporary matrix for early healing promoting factors and supports the infiltration of cells.[34]

Subsequently, mobilized neutrophils and macrophages release growth factors into the matrix. Importantly, several angiogenic growth factors, such as VEGF-A, FGF-2, and PDGF-BB can bind to fibrin and fibronectin and thereby render the wound matrix a growth factor presentation platform.[35-39] This angio-competent milieu attracts endothelial, perivascular, and stromal cells that, by secretion of cellular fibronectin and fibrillar collagen, remodel the initial fibrin clot into a provisional matrix. During tube formation, endothelial and perivascular cell types start to lay ECM components around the blood vessels and thereby establish the basement membrane of mature blood vessels. The main components of the basement membrane are collagen type IV, laminins, nidogens, fibronectin, and perlecan. However, many more ECM components, such as collagen type XV and XVIII and glycosaminoglycans contribute to the basement membrane.[40, 41] Besides providing structural and physical support to cells, the vascular ECM acts as a pro-angiogenic signal transducer. The ECM composition itself exerts an instructive role for vascular development covering, e.g., early endothelial lumen formation, endothelial quiescence, and mature vessel stabilization.[10, 40, 42, 43] On the other hand, the anti-angiogenic effects of collagen IV, XV, and XVIII cleavage products actively regulate blood vessel formation and stability.[40, 44] Since neovascularization is strongly associated with the dynamic remodeling of the ECM, enzymes that disintegrate ECM components are key regulators of neovascularization. Plasmin and the members of the large matrix metalloproteinase (MMP) family are proteases able to cleave ECM proteins. This proteolytic ECM breakdown enables three fundamental processes: 1) disintegration of the basement membrane and cell-cell junctions resulting in breakout of endothelial and perivascular cells, 2) liberation of angiogenic growth factors from their ECM storage, and 3) turnover and maturation of the ECM during vessel formation and maturation. But, to achieve a strictly controlled degradability-stability equilibrium, the proteolytic activity of both plasmin and MMPs is controlled by direct counterparts, such as alpha 2-antiplasmin and tissue inhibitors of metalloproteinases (TIMP).

5.0 Hydrogels for Vascular Engineering

Vascular-deficient tissues can be treated by either the delivery of angiogenic growth factors or the transplantation of pre-vascularized tissues. While the delivery of growth factors builds on the individual´s endogenous vascularization potential, the transplantation of pre-vascularized tissues is applicable even if the wound-healing cascade is strongly impaired. Looking at the natural processes of blood vessel development and regeneration, vascular engineering minimally requires angiogenic growth factors or vascular cells in combination with adequate 3D scaffolds (Fig. 1B). Although completely scaffold-free strategies exist (e.g., spheroid or cell sheet technology[45-47]), most vascular engineering strategies require appropriate scaffolds that enable the transport of growth factors or cells and provide a 3D matrix for blood vessel (in)growth and formation (Fig. 1C).

Hydrogel materials have become valuable 3D scaffolds for vascular engineering since they share many features with the natural ECM, including high water content and viscoelastic properties. Naturally occurring or synthetic polymers that are hydrophilic and form interconnected networks by physical interactions or chemical bonds can form hydrogels. By controlling the chemical properties of the polymer, its initial concentration and the distance between molecular interactions in the network, hydrogel parameters, such as stiffness, swelling, and pore size can be readily adapted.[48, 49] To date, a variety of biocompatible, natural, or synthetic ECM-mimicking hydrogels are commercially available for a broad spectrum of users (see Caliari and Burdick[50]). Many of these hydrogel systems can be used to engineer pre-vascularized tissue constructs in vitro prior to in vivo implantation by drawing the evolutionary memory of endothelial cells to assemble into micro-capillary networks when co-cultured with perivascular or other mesenchymal cells.

5.1 Natural ECM hydrogels and modified ECM protein-based (semi-synthetic) hydrogels

The main class of hydrogel materials directly derived from natural ECM is protein polymers. Protein polymers' great advantage is their intrinsic bioactivity, which includes the provision of cell adhesion and proteolytic degradable sites. Moreover, the application of ECM protein polymers has a long tradition in vascular biology and existing knowledge of this materials class is profound. Pioneering work in the 1980s from Nicosia et al. and Montesano et al. showed that fibrin and collagen type I hydrogels can host micro-capillary-like structures ex vivo.[51-55] Based on this early work, ECM protein-based hydrogels have become the most widely used scaffold materials for vascular engineering. The first matrix vascular cells encounter during vascularization in vivo is fibrin. Fibrin hydrogels result from the enzymatic activation of fibrinogen by thrombin, followed by terminal factor XIIIa-mediated, enzymatic cross-linking. In vitro, fibrin hydrogels are suitable templates for endothelial and mesenchymal cells, which under appropriate co-culture conditions readily form micro-capillaries. Studies from different labs have proven that such in vitro established pre-vascularized fibrin constructs connect (inosculate) to the host vasculature when transplanted into mice.[56-60] Importantly, one study provided experimental evidence for the general assumption that an in vitro pre-vascularization step is indeed beneficial over only cell delivery (Fig. 2).[56] Collagen type I is part of the interstitial ECM and has the intrinsic capacity to self-assemble into hydrogels under physiological conditions. In a seminal series of in vitro studies, Davis and co-workers have shown that collagen hydrogels allow for the formation of 3D micro-capillary networks by endothelial and perivascular cells.[61-65] Other studies showed that capillaries engineered within collagen hydrogels successfully inosculate after in vivo implantation.[66-70] However, native collagen hydrogels might miss the optimal degradability-stability equilibrium since the low collagen concentrations suitable for 3D culture of micro-capillary networks result in mechanically weak scaffolds. To overcome this dilemma, collagen hydrogels have been modified by plastic compression[71-73] or by chemical modifications as discussed below.

To combine their cell-instructive and biocompatible features with desired mechanical properties, natural ECM components can be tuned by chemical modifications. The resulting semi-synthetic biomaterials are beneficial, especially when using highly proteolytic cells as is the case in vascular engineering. In this regard, a photo-cross-linkable collagen-poly(ethylene glycol) (PEG) hybrid material, in which the mechanical properties could be increased independently of the material density, has been developed.[74] Within this collagen-PEG hydrogel micro-capillary networks can be generated. Another promising and widely used ECM-based material is gelatin, which is derived from collagens by hydrolytic breakage. Like its mother material, gelatin can form physical hydrogels of quite weak mechanical properties. Unfortunately, gelatin hydrogels are just stable below 37 °C, which makes them inappropriate as independent scaffolds. However, methacrylic anhydride modification renders gelatin into photo-cross-linkable gelatin methacrylate (GelMA) hydrogels of excellent tissue engineering properties.[75-77] Using GelMA as vascularization scaffold, micro-capillary networks that anastomose with a murine host can be achieved either through in vitro pre-vascularization or subcutaneous injection of polymerizing hydrogel precursors and vascular cells.[78, 79] These studies clearly demonstrate the benefits of chemically modified ECM polymers because the bioactivity of gelatin allowed for micro-capillary network formation and the photo-cross-link mechanism for stable material properties.

5.2 Modified GAG-based (semi-synthetic) hydrogels and PEG-based (synthetic) hydrogels

While natural and chemically modified ECM protein-based hydrogels are excellent substrates for the culture of vascularized tissues, they exhibit inherent materials properties. To gain further control over materials properties, carbohydrate-based materials, such as glycosaminoglycans (GAG) serve as the basis for hydrogel engineering. Upon modification with cross-linkable chemical groups, GAG hydrogels can be produced with defined physical properties. Although GAGs as components of the natural ECM are known to be degraded by enzymes, to bind growth

factors and to interact with cell-surface receptors, their modification with additional biologically functional modules, such as small peptides or protein domains is key for vascular engineering. As we can learn from hyaluronic acid (HA), two biological features from the natural ECM are required to turn these semi-synthetic scaffolds into cell-friendly hydrogels for neovascularization: sites for integrin-dependent cell adhesion and sites for proteolytic remodeling. Indeed, acrylated HA when modified with the cell-adhesion peptide arginine–glycine–aspartic acid (RGD) and polymerized by a thiol-cross-linker containing MMP-sensitive peptides results in a bioactive hydrogel, which allows cell adhesion and proteolytic remodeling.[80] These HA hydrogel modifications are sufficient to enable in vitro formation of micro-capillary networks that upon in vivo transplantation anastomose with the host vasculature.[81-84] Not surprisingly, hybrid hydrogels built by the cross-linking of the GAG heparin and poly(ethylene glycol) (PEG) can host micro-capillary networks when biologically modified by RGD and MMP-sensitivity.[85, 86] However, in contrast to HA hydrogels, heparin-based hydrogels through the high abundance of sulfate groups and the resulting negative charge enables the affinity binding of various growth factors.

We have seen that hydrogels derived from naturally occurring ECM components are suitable scaffolds for vascular engineering, and that they can be engineered within a certain range towards desired functions. Reverse engineered synthetic hydrogels aim to mimic the natural ECM but rely on fully controlled design and synthesis.[87-90] Since manufactured from scratch, synthetic hydrogel materials can be seen as blank slates free of animal products and confounding biological factors, per se. Conceivably, in these initially cell-inert synthetic materials biological modifications are essential to render them cell-friendly and biologically functional. Interestingly, it seems that the minimalistic approach of facilitating cell adhesion by RGD and matrix remodeling by MMP-sensitive peptides is generally sufficient for micro-capillary network formation in synthetic hydrogels. Although these materials are still less used than natural ECM hydrogels, some work proving the potential of synthetic hydrogels for

vascular engineering has been reported within the last decade. Hydrogels exclusively composed of PEG allow neovascularization in vitro and in vivo when RGD sites and MMP-sensitivity optimized the material. In this regard, several studies showed that co-culturing of endothelial and mesenchymal cells in PEG hydrogels is a promising approach for the formation of 3D pre-vascularized scaffolds with fully defined properties.[91-94]

5.3 Controlled delivery of vascular endothelial growth factor by hydrogel immobilization

In vitro engineered vascular networks hold great promise to become transplantable functional tissue units. However, their production relies on the expansion of autologous cells under complex production environments and is labor- and cost-intensive. By contrast, the concept of therapeutic angiogenesis builds on the idea that upon proper stimulation with key pro-angiogenic factors, the body´s innate angiogenic program initiates the formation of new vessels. Early therapeutic angiogenesis attempts, conducted by the infusion of plasmid DNA encoding the expression VEGF or recombinant VEGF proteins into various tissue sites as well as into blood vessels, showed promising results in preclinical studies that could not be reproduced in clinical trials.[95] To improve therapeutic angiogenesis by at least partially localized delivery, hydrogels with no or weak affinities for growth factors were employed for release by passive or slowed-down diffusion.[96] In recent years, multiple sophisticated strategies that enable the tailoring of growth factor release towards specific requirements have been developed (for a comprehensive review see Briquez et al. [97]). Here, we look at examples of VEGF delivery to demonstrate how hydrogel and growth factor engineering can boost the response of angiogenic growth factors.

An elegant strategy to functionalize fibrin hydrogels with growth factors has been developed by emulating the factor XIII (FXIII)-mediated cross-linking taking place in native fibrin. In detail, a short peptide sequence (NQEQVSPL) responsible for linking α2-plasmin inhibitor to fibrin allows the covalent XIIIa-mediated binding of growth factors to fibrin when fused to the

N-terminus of growth factors or peptides.[98, 99] Resulting growth factor-modified fibrin hydrogels enable the release of their growth factor payload upon cell-mediated matrix degradation. Applying this engineering approach in vivo resulted in an improved angiogenic performance of fibrin-immobilized as compared to freely diffusible $VEGF_{121}$ (Fig. 3A).[100-102] However, a considerable downside of fibrin hydrogels is their relatively fast degradation kinetics in vivo, resulting in the robust induction of new vessels that are not stabilized and regress due to the short duration of VEGF-treatment.[102] Although this material´s degradability-stability equilibrium related problem could be partially addressed by changing the fibrin amount, conceivably a higher material density is negatively correlated with vascular morphogenesis.[103, 104] Therefore, degradation-resistant and VEGF-releasing fibrin hydrogels were engineered by modifying fibrin with the covalently bound fibrinolysis inhibitor aprotinin and $VEGF_{164}$ at the same time (Fig. 3B). These long-lasting fibrin hydrogels enable a cell-demanded, continuous, and slow release of $VEGF_{164}$ and were shown to induce the formation of lasting blood vessels in skeletal muscles and to improve the perfusion of ischemic hind-limbs and skin flaps in mice.[105]

Another way of controlling the stability of growth factor releasing hydrogels is to engineer them from the bottom-up using defined components. The degradability of synthetic PEG hydrogels, for instance, can be tailored by introducing peptide sequences with variable sensitivities for the proteolytic degradation by MMPs and Plasmin.[106, 107] For in situ vascularization, different VEGF-variants were immobilized to cell-free, MMP-sensitive PEG hydrogels and liberated by host cells.[108, 109] These studies showed that for synthetic hydrogels, bound VEGF outperformed soluble VEGF in inducing neovascularization in vivo in a qualitative as well as quantitative way.

Current efforts to immobilize growth factors to hydrogels are directed towards more flexible engineering strategies. On the one hand, the binding of various native growth factors could be achieved by modifying hydrogels, for example with fibronectin domains, fibrinogen derived

peptides, or heparin.[110-112] On the other hand, growth factors were engineered with affinities such that they bind to hydrogels comprising ECM-components, streptavidin, or barnase.[113-115]

5.4 Limitations of current hydrogel-based vascularization strategies

Despite tremendous progress in hydrogel-based vascularization strategies, major problems have emerged from the application of hydrogels as scaffolds for vascularized tissues. So far, except for skin, which is a relatively thin tissue, only small-size constructs have been successfully pre-vascularized and implanted in vivo, due mostly to limitations in fabrication techniques and the soft material properties of cell-friendly hydrogels. Nevertheless, soft hydrogels can be applied as a gelatinous cell carrier solution within porous and solid scaffolds. For instance, endothelial and perivascular cells can be suspended in fibrinogen or matrigel solutions and poured into macroporous sponges prior to gel polymerization. Applying this technique in poly(lactic-co-glycolic acid)/poly(L-lactic acid) co-polymers-based sponges facilitated the establishment of pre-vascularized capillary networks, which were shown to anastomose in vivo.[116, 117] Although overcoming the stability problems of soft hydrogels, such a scaffold-in-scaffold strategy still relies on the "spontaneous" self-assembly of mono-dispersed cell mixtures, which per default result in micro-capillaries with diameters of about 10 μm. However, clinical relevant tissue constructs of large size require large-caliber vessels as well.

For therapeutic angiogenesis, a substantial repertoire of angiogenic growth factors is known today. However, knowledge on the spatiotemporal availability and dynamics of growth factors (often described as 4^{th} dimension) in tissues is still quite limited and remains a major challenge. Although it is widely accepted that growth factors appear in gradients resulting from diffusion and binding to the ECM, the characteristics of these growth factor gradients are highly complex, tissue-specific, and therefore difficult to determine. Furthermore, for the engineering of specific tissue factors controlling vascularization as well as factors directing differentiation need to be

present at the same time. Hence, potential cross-talks between angiogenic and tissue specific signals and cells need to be considered.

6.0 Novel (hydrogel-based) Technologies for Vascularized Tissue Models

In vascular biology, much knowledge has been generated using various well established in vivo evaluations and transgenic animal models.[118, 119] Additionally, cellular and molecular mechanism controlling capillary architecture, morphogenesis, and EC-perivascular cell communication have been dissected in 3D in vitro systems using natural ECM hydrogels. Furthermore, engineered micro-capillary networks or molecules with blood vessel-modulating functions have been delivered in vivo using hydrogels. Yet, large caliber vessels are still missing in current pre-vascularization systems. Furthermore, while angiogenesis inhibitors proved successful for the treatment of ocular vascular diseases, their use for cancer treatments in many instances did not lead to the targeted effects.[120, 121]

The study of basic vascular biology, the development of novel therapeutic strategies, and the establishment of in vitro platforms for personalized therapies will benefit from physiologically relevant and reproducible 3D tissue models. Since novel materials and manufacturing technologies are key to achieve such models, we will discuss their impact on the generation of vascularized tissue models in the next sections.

6.1 Synthetic hydrogel materials to study basic vascular biology

The American Heart Association states that collagen type I and fibrin hydrogels are the gold standard materials for 3D in vitro vascular biology assays.[122] Indeed, these systems have provided invaluable insight into the morphogenesis of blood vessel capillaries. However, biological outcomes might be influenced by ECM hydrogel properties. In contrast, semi-synthetic and synthetic hydrogels enable study of the function of ECM components on vascular morphogenesis and signaling in the absence of confounding ECM signals. Such defined

conditions will further allow control over growth factor presentation and the evaluation of growth factor gradients, for example during sprouting angiogenesis and pericyte recruitment.[123] The in vitro recapitulation of angiogenic processes under controlled matrix and growth factor presentation conditions will be important to the study of spontaneous morphogenic processes and associated molecular and biochemical functions. A recent publication shows the adequacy of fully synthetic hydrogels to provide insights into the heterocellular communication between perivascular and endothelial cells.[124] Moreover, in synthetic hydrogels established vascular models could provide a platform to manipulate morphogenic processes by using sophisticated and ideally remotely controllable cells and materials components as they are becoming available.[125, 126] For example, cells that upon chemical-, temperature-, or light-mediated activation induce the expression of angiogenic stimuli, receptors, or even cell-cell adhesion molecules could be used to study cellular communication during vessel formation and remodeling. Furthermore, hydrogels whose stiffness, cell adhesion, or growth factor binding properties can be modulated by light could serve to control the local activation of sprouting angiogenesis.

6.2 Vascularized models of specific types of tissues

Vessel properties vary among different tissues and reciprocally vessels can influence the development and function of tissues. Therefore, vessel functions should be studied in tissue specific models consisting of multiple properly arranged and differentiated cell types. Since they are relatively simple in structure, dermo-epidermal tissue models were among the first engineered differentiated tissues with an established functional vascularization.[58, 59] However, in tissues with high structural complexity, the localization of growth factor stimulation relies on a spatial patterning to prevent a potential interference of different signaling cues. The need for the spatial separation of growth factors has been shown in engineered bone and bone marrow mimicking environments, as they require at least two different growth factors.[94] While the

osteogenic signal BMP-2 directed mesenchymal progenitor cells towards osteogenic commitment, FGF-2 supported the maintenance of an undifferentiated phenotype. Since in this system both factors promote the formation of a 3D micro-capillary network, the localized presentation of the factors could be used to study vascular functions responsible for the maintenance of hematopoietic stem cells in their bone marrow compartment.

6.3 Vascularized cancer models

Cancer is known to continuously promote angiogenesis and to benefit from excessive vascularization. Therefore, the inhibition of angiogenesis was considered as a strategy to reduce cancer progression. Surprisingly, the treatment of cancer patients with VEGF inhibitors in many instances did result only in the minimal extension of progression free survival.[127] The cause of limited treatment efficiency can be manifold and includes the compensation of treatment effects through non-angiogenic mechanisms. Nevertheless, mechanisms also related to angiogenesis, such as the co-option of preexisting vessel structures or the compensation for the lack of one angiogenic cue by the upregulation of another cue, are currently seen as important aspects to be carefully studied. Moreover, within the last decade, the dogma of vascularization-based cancer treatment has shifted from the inhibition of blood vessel formation to a normalization of the structurally and functionally aberrant cancer induced blood vessels.[128] Due to the enormous need for meaningful in vitro screening assays, 3D models of cancer have become one of the fastest growing research topics in both cancer biology and tissue engineering. Since angiogenesis is one of the hallmarks of cancer, vascularization has been integrated into engineered 3D cancer models. While first vascularized cancer models were established in naturally occurring ECM hydrogels, models engineered in semi-synthetic and synthetic hydrogels are now becoming available and have the potential to give rise to highly structured, vascularized tumor environments.[129-133]

6.4 Manufacturing channels in hydrogels to engineer large caliber vessels

Vascular engineering can benefit from novel biofabrication techniques, such as bioprinting, micro-molding and lithography, in moving towards the development of larger diameter vessels. By applying these techniques, hydrogel materials that contain imbedded channels of several hundred micrometers can be obtained. Subsequently, the created channels can be lined by endothelial cells and can serve as potential access sites for perfusion. For example, Bertassoni et al. have molded channels into stiff GelMA and PEG hydrogels using agarose rods and seeded the channels with endothelial cell suspensions after removing the rods.[134] In two other studies, gold rods coated with oligopeptides and ECs have been used to couple channel creation and endothelialization. After hydrogel formation, the endothelial cells were electrochemically transferred from the gold rod to the hydrogel and thereby formed an endothelialized channel.[135, 136] Another strategy to establish endothelialized channels is to combine micro-molding and additive manufacturing as excellently shown in collagen hydrogels.[137, 138] The general concept of leaving behind a channel in hydrogels can also be implemented by printing temperature dissolvable materials into temperature stable hydrogels and release them on demand by an adequate temperature shift.[139-141] To save labor, channels can be created in hydrogels by laser-based photo-ablation of the bulk hydrogel material, a technique that renounces molding and allows for high spatiotemporal control over the channel design.[142] Independent of the fabrication method and the employed material, the channel-based vascularization concept holds great potential for tissue engineering applications. But, the transfer of big channels to in vivo applications remains to be realized.

6.5 Microfluidic technology for the perfusion of vascularized tissue models

Today, most vascularization models are still based on non-perfused micro-capillary networks. Factors that control flow-mediated vessel maturation and remodeling are not accessible in non-perfused systems and consequently cannot be studied. Additionally, drugs distributed using the

vasculature cannot be the tested in non-perfused systems. Therefore, novel vascularization models build on the formation of 3D vascular structures in microfluidic devices, which can be perfused with cell culture medium and permit the testing of pro- and anti-angiogenic compounds. Large channels can be formed, as we discussed in section 6.4, and connected to microfluidic devices. When such microfluidic channels are fused with micro-capillary networks the whole engineered vascularized model can be perfused.[143-146] Conceivably, the dominant field in microfluidics perfused tissue models is cancer.[147] In this regard, vascularized microtumors (VMT) have been generated in hydrogels.[148, 149] When such VMT models were connected to perfusion, they allowed for the efficient screening of anti-cancer drugs that are affecting the microtumors as well as the tumor vasculature (Fig. 4). Furthermore, the combination of large vascular structures with self-arranged micro-capillaries would allow for the formation of hierarchically organized vascular trees.

6.6 Outlook: Standardized platforms for drug screening and personalized medicine

The above-described approaches to engineer vessel models are mainly designed to gain insight into physiological and pathological tissue functions. By using human cells, engineered vessel models avoid species-related false positive and negative results and therefore are interesting alternatives to preclinical animal models. However, to speed up the development of novel vascular drugs, the integration of vessel models with reproducible high throughput platforms is required. The potential benefit of such platforms was recently shown by using induced pluripotent stem cells and PEG hydrogels. [150] Since similar assays could be run with adult stem or induced pluripotent stem cells derived from healthy or diseased human individuals, these assays can also help to bring forward personalized medicine in the field of vascular diseases.

7. Summary

Engineered pre-vascularized tissues and the delivery of angiogenesis promoting or inhibiting therapeutics are promising approaches to treat diseases related to malfunction of the vasculature. Additionally, emerging vascularized tissue models (summarized in Fig. 5) enable the study of basic vessel biology under near-physiological and pathology mimicking conditions in vitro. If scalable, vascularized tissue models would allow screening for therapeutic compounds under controlled and highly standardized conditions. The development of next-generation vascularized tissues critically depends on hydrogel systems tailorable towards cell-specific functions and compatible with innovative (micro)-manufacturing technologies. Therefore, the development of novel clinically translatable vascular treatments as well as personalized tissue models require the continuous integration of knowhow from basic vascular biology, hydrogel engineering, biofabrication, and medical needs.

Take home messages

- While pro- and anti-angiogenic treatments have shown promising results in basic research, clinical trials have not met expectations
- ECM-inspired hydrogels are scaffold materials allowing for the engineering of vascularized tissues.
- The main limitation of engineered pre-vascularized tissues is the lack of large vessels.
- Physiologically relevant vascularized tissue models will enable the study of diseases as well as the testing of therapeutics under controlled in vitro conditions.
- Hydrogel systems with defined and tunable materials properties are key for the development of innovative 3D tissue models.
- Future personalized, vascularized tissues and disease models depend on the continuous integration of knowhow from basic vascular biology, hydrogel engineering, biofabrication, and medical needs.

Acknowledgment and funding sources:

This work has been supported by the European Union's Seventh Framework Programme (iTERM grant agreement No. 607868) and by the Swiss National Science Foundation (310030-169808/1).

Author disclosure and ghostwriting: No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

About the authors: Ulrich Blache is a graduate student in the Department of Health Sciences and Technology of the ETH Zurich and is employed at the University Hospital Zurich. He works on the integration of vascular cell biology and synthetic hydrogels to engineer defined 3D models of the micro-capillary bed. Martin Ehrbar, Ph.D., is head of research at the Department of Obstetrics of the University Hospital Zurich. He leads the Laboratory for Cell and Tissue Engineering and focuses on the combination of materials engineering with cell biology and clinical research. His core competence is the integration of modular synthetic materials with stem cell biology to design novel healing models in vitro and in vivo.

Abbreviations and acronyms:

3D = three dimensional

ANG-1 / ANG-2 = Angiopoietin 1 / 2

BMP-4 / BMP-2 = bone morphogenetic protein 2 / 4

DLL-4 = delta-like ligand 4

EC = endothelial cell

ECM = extracellular matrix

FGF-2 = fibroblast growth factor 2

FXIII = blood coagulation factor XIII

GAG = glycosaminoglycan

GelMA = photo-cross-linkable gelatin methacrylate

HA = hyaluronic acid

MMP = matrix metalloproteinase

PDGF-BB = platelet derived growth factor BB

PEG = poly(ethylene glycol)

RGD = cell adhesion peptide: arginine–glycine–aspartic acid

SDF-1α = stromal cell-derived factor 1α

TGF-β = Transforming growth factor β

TIMP = tissue inhibitors of metalloproteinases

VEGF-A = vascular endothelial growth factor A

VEGFR = vascular endothelial growth factor receptor

VMT = vascularized microtumors

References:

1. Potente M, Makinen T: Vascular heterogeneity and specialization in development and disease. *Nat Rev Mol Cell Biol.* 2017;18:477-494.

2. Asahara T, Masuda H, Takahashi T, et al.: Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circulation research.* 1999;85:221-228.

3. Yoder MC: Human endothelial progenitor cells. *Cold Spring Harb Perspect Med.* 2012;2:a006692.

4. Drake CJ: Embryonic and adult vasculogenesis. *Birth Defects Res C Embryo Today.* 2003;69:73-82.

5. Marcelo KL, Goldie LC, Hirschi KK: Regulation of endothelial cell differentiation and specification. *Circulation research.* 2013;112:1272-1287.

6. Djonov V, Schmid M, Tschanz SA, Burri PH: Intussusceptive angiogenesis: its role in embryonic vascular network formation. *Circulation research.* 2000;86:286-292.

7. Djonov V, Baum O, Burri PH: Vascular remodeling by intussusceptive angiogenesis. *Cell Tissue Res.* 2003;314:107-117.
8. Ribatti D, Crivellato E: "Sprouting angiogenesis", a reappraisal. *Dev Biol.* 2012;372:157-165.
9. Burri PH, Hlushchuk R, Djonov V: Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. *Dev Dyn.* 2004;231:474-488.
10. Senger DR, Davis GE: Angiogenesis. *Cold Spring Harb Perspect Biol.* 2011;3:a005090.
11. Sundberg C, Nagy JA, Brown LF, et al.: Glomeruloid Microvascular Proliferation Follows Adenoviral Vascular Permeability Factor/Vascular Endothelial Growth Factor-164 Gene Delivery. *The American journal of pathology.* 2001;158:1145-1160.
12. Carmeliet P, Jain RK: Molecular mechanisms and clinical applications of angiogenesis. *Nature.* 2011;473:298-307.
13. Krock BL, Skuli N, Simon MC: Hypoxia-induced angiogenesis: good and evil. *Genes Cancer.* 2011;2:1117-1133.
14. Jakobsson L, Franco CA, Bentley K, et al.: Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol.* 2010;12:943-953.
15. Phng LK, Gerhardt H: Angiogenesis: a team effort coordinated by notch. *Dev Cell.* 2009;16:196-208.
16. Armulik A, Genove G, Betsholtz C: Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell.* 2011;21:193-215.
17. Gaengel K, Genove G, Armulik A, Betsholtz C: Endothelial-mural cell signaling in vascular development and angiogenesis. *Arteriosclerosis, thrombosis, and vascular biology.* 2009;29:630-638.
18. Crisan M, Chen CW, Corselli M, Andriolo G, Lazzari L, Peault B: Perivascular multipotent progenitor cells in human organs. *Ann N Y Acad Sci.* 2009;1176:118-123.
19. Crisan M, Yap S, Casteilla L, et al.: A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell.* 2008;3:301-313.
20. Sacchetti B, Funari A, Michienzi S, et al.: Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell.* 2007;131:324-336.
21. Feng J, Mantesso A, De Bari C, Nishiyama A, Sharpe PT: Dual origin of mesenchymal stem cells contributing to organ growth and repair. *Proceedings of the National Academy of Sciences of the United States of America.* 2011;108:6503-6508.
22. Putnam AJ: The Instructive Role of the Vasculature in Stem Cell Niches. *Biomater Sci.* 2014;2:1562-1573.
23. Billaud M, Donnenberg VS, Ellis BW, et al.: Classification and Functional Characterization of Vasa Vasorum-Associated Perivascular Progenitor Cells in Human Aorta. *Stem Cell Reports.* 2017;9:292-303.
24. Guimaraes-Camboa N, Cattaneo P, Sun Y, et al.: Pericytes of Multiple Organs Do Not Behave as Mesenchymal Stem Cells In Vivo. *Cell Stem Cell.* 2017;20:345-359.e345.
25. Korn C, Augustin HG: Mechanisms of Vessel Pruning and Regression. *Dev Cell.* 2015;34:5-17.
26. Kochhan E, Lenard A, Ellertsdottir E, et al.: Blood flow changes coincide with cellular rearrangements during blood vessel pruning in zebrafish embryos. *PloS one.* 2013;8:e75060.
27. Ricard N, Simons M: When it is better to regress: dynamics of vascular pruning. *PLoS Biol.* 2015;13:e1002148.

28. le Noble F, Moyon D, Pardanaud L, et al.: Flow regulates arterial-venous differentiation in the chick embryo yolk sac. *Development (Cambridge, England).* 2004;131:361-375.
29. Udan RS, Vadakkan TJ, Dickinson ME: Dynamic responses of endothelial cells to changes in blood flow during vascular remodeling of the mouse yolk sac. *Development (Cambridge, England).* 2013;140:4041-4050.
30. Chen Q, Jiang L, Li C, et al.: Haemodynamics-driven developmental pruning of brain vasculature in zebrafish. *PLoS Biol.* 2012;10:e1001374.
31. Lenard A, Daetwyler S, Betz C, et al.: Endothelial cell self-fusion during vascular pruning. *PLoS Biol.* 2015;13:e1002126.
32. Simonavicius N, Ashenden M, van Weverwijk A, et al.: Pericytes promote selective vessel regression to regulate vascular patterning. *Blood.* 2012;120:1516-1527.
33. Korn C, Scholz B, Hu J, et al.: Endothelial cell-derived non-canonical Wnt ligands control vascular pruning in angiogenesis. *Development (Cambridge, England).* 2014;141:1757-1766.
34. van Hinsbergh VW, Collen A, Koolwijk P: Role of fibrin matrix in angiogenesis. *Ann N Y Acad Sci.* 2001;936:426-437.
35. Sahni A, Altland OD, Francis CW: FGF-2 but not FGF-1 binds fibrin and supports prolonged endothelial cell growth. *Journal of thrombosis and haemostasis : JTH.* 2003;1:1304-1310.
36. Sahni A, Francis CW: Vascular endothelial growth factor binds to fibrinogen and fibrin and stimulates endothelial cell proliferation. *Blood.* 2000;96:3772-3778.
37. Sahni A, Odrljin T, Francis CW: Binding of basic fibroblast growth factor to fibrinogen and fibrin. *The Journal of biological chemistry.* 1998;273:7554-7559.
38. Peng H, Sahni A, Fay P, et al.: Identification of a binding site on human FGF-2 for fibrinogen. *Blood.* 2004;103:2114-2120.
39. Wijelath ES: Novel Vascular Endothelial Growth Factor Binding Domains of Fibronectin Enhance Vascular Endothelial Growth Factor Biological Activity. *Circulation research.* 2002;91:25-31.
40. Kalluri R: Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat Rev Cancer.* 2003;3:422-433.
41. Yurchenco PD: Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harb Perspect Biol.* 2011;3.
42. Davis GE, Senger DR: Endothelial extracellular matrix: biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. *Circulation research.* 2005;97:1093-1107.
43. Davis GE, Stratman AN, Sacharidou A, Koh W: Molecular basis for endothelial lumen formation and tubulogenesis during vasculogenesis and angiogenic sprouting. *International review of cell and molecular biology.* 2011;288:101-165.
44. Mott JD, Werb Z: Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol.* 2004;16:558-564.
45. Alajati A, Laib AM, Weber H, et al.: Spheroid-based engineering of a human vasculature in mice. *Nat Methods.* 2008;5:439-445.
46. Asakawa N, Shimizu T, Tsuda Y, et al.: Pre-vascularization of in vitro three-dimensional tissues created by cell sheet engineering. *Biomaterials.* 2010;31:3903-3909.
47. Nishiguchi A, Matsusaki M, Asano Y, Shimoda H, Akashi M: Effects of angiogenic factors and 3D-microenvironments on vascularization within sandwich cultures. *Biomaterials.* 2014;35:4739-4748.

48. Peppas NA, Hilt JZ, Khademhosseini A, Langer R: Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. *Advanced Materials.* 2006;18:1345-1360.
49. Kirschner CM, Anseth KS: Hydrogels in Healthcare: From Static to Dynamic Material Microenvironments. *Acta Mater.* 2013;61:931-944.
50. Caliari SR, Burdick JA: A practical guide to hydrogels for cell culture. *Nat Methods.* 2016;13:405-414.
51. Nicosia RF, Madri JA: The microvascular extracellular matrix. Developmental changes during angiogenesis in the aortic ring-plasma clot model. *The American journal of pathology.* 1987;128:78-90.
52. Nicosia RF, Tchao R, Leighton J: Histotypic angiogenesis in vitro: light microscopic, ultrastructural, and radioautographic studies. *In vitro.* 1982;18:538-549.
53. Nicosia RF, Tchao R, Leighton J: Interactions between newly formed endothelial channels and carcinoma cells in plasma clot culture. *Clinical & experimental metastasis.* 1986;4:91-104.
54. Montesano R, Mouron P, Orci L: Vascular outgrowths from tissue explants embedded in fibrin or collagen gels: a simple in vitro model of angiogenesis. *Cell biology international reports.* 1985;9:869-875.
55. Montesano R, Pepper MS, Vassalli JD, Orci L: Phorbol ester induces cultured endothelial cells to invade a fibrin matrix in the presence of fibrinolytic inhibitors. *Journal of cellular physiology.* 1987;132:509-516.
56. Chen X, Aledia AS, Ghajar CM, et al.: Prevascularization of a fibrin-based tissue construct accelerates the formation of functional anastomosis with host vasculature. *Tissue engineering. Part A.* 2009;15:1363-1371.
57. Chen X, Aledia AS, Popson SA, Him L, Hughes CC, George SC: Rapid anastomosis of endothelial progenitor cell-derived vessels with host vasculature is promoted by a high density of cotransplanted fibroblasts. *Tissue engineering. Part A.* 2010;16:585-594.
58. Klar AS, Guven S, Biedermann T, et al.: Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells. *Biomaterials.* 2014;35:5065-5078.
59. Marino D, Luginbuhl J, Scola S, Meuli M, Reichmann E: Bioengineering dermo-epidermal skin grafts with blood and lymphatic capillaries. *Science translational medicine.* 2014;6:221ra214.
60. Montano I, Schiestl C, Schneider J, et al.: Formation of human capillaries in vitro: the engineering of prevascularized matrices. *Tissue engineering. Part A.* 2010;16:269-282.
61. Bayless KJ, Davis GE: The Cdc42 and Rac1 GTPases are required for capillary lumen formation in three-dimensional extracellular matrices. *Journal of cell science.* 2002;115:1123-1136.
62. Kamei M, Saunders WB, Bayless KJ, Dye L, Davis GE, Weinstein BM: Endothelial tubes assemble from intracellular vacuoles in vivo. *Nature.* 2006;442:453-456.
63. Stratman AN, Davis MJ, Davis GE: VEGF and FGF prime vascular tube morphogenesis and sprouting directed by hematopoietic stem cell cytokines. *Blood.* 2011;117:3709-3719.
64. Stratman AN, Malotte KM, Mahan RD, Davis MJ, Davis GE: Pericyte recruitment during vasculogenic tube assembly stimulates endothelial basement membrane matrix formation. *Blood.* 2009;114:5091-5101.

65. Stratman AN, Schwindt AE, Malotte KM, Davis GE: Endothelial-derived PDGF-BB and HB-EGF coordinately regulate pericyte recruitment during vasculogenic tube assembly and stabilization. *Blood.* 2010;116:4720-4730.
66. Black AF, Berthod F, L'Heureux N, Germain L, Auger FA: In vitro reconstruction of a human capillary-like network in a tissue-engineered skin equivalent. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 1998;12:1331-1340.
67. Shepherd BR, Chen HY, Smith CM, Gruionu G, Williams SK, Hoying JB: Rapid perfusion and network remodeling in a microvascular construct after implantation. *Arteriosclerosis, thrombosis, and vascular biology.* 2004;24:898-904.
68. Koike N, Fukumura D, Gralla O, Au P, Schechner JS, Jain RK: Tissue engineering: creation of long-lasting blood vessels. *Nature.* 2004;428:138-139.
69. Au P, Tam J, Fukumura D, Jain RK: Bone marrow-derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. *Blood.* 2008;111:4551-4558.
70. White SM, Pittman CR, Hingorani R, et al.: Implanted cell-dense prevascularized tissues develop functional vasculature that supports reoxygenation after thrombosis. *Tissue engineering. Part A.* 2014;20:2316-2328.
71. Brown RA, Wiseman M, Chuo CB, Cheema U, Nazhat SN: Ultrarapid Engineering of Biomimetic Materials and Tissues: Fabrication of Nano- and Microstructures by Plastic Compression. *Advanced functional materials.* 2005;15:1762-1770.
72. Cheema U, Brown RA: Rapid Fabrication of Living Tissue Models by Collagen Plastic Compression: Understanding Three-Dimensional Cell Matrix Repair In Vitro. *Adv Wound Care (New Rochelle).* 2013;2:176-184.
73. Alekseeva T, Unger RE, Brochhausen C, Brown RA, Kirkpatrick JC: Engineering a microvascular capillary bed in a tissue-like collagen construct. *Tissue engineering. Part A.* 2014;20:2656-2665.
74. Singh RK, Seliktar D, Putnam AJ: Capillary morphogenesis in PEG-collagen hydrogels. *Biomaterials.* 2013;34:9331-9340.
75. Van Den Bulcke AI, Bogdanov B, De Rooze N, Schacht EH, Cornelissen M, Berghmans H: Structural and rheological properties of methacrylamide modified gelatin hydrogels. *Biomacromolecules.* 2000;1:31-38.
76. Benton JA, DeForest CA, Vivekanandan V, Anseth KS: Photocrosslinking of gelatin macromers to synthesize porous hydrogels that promote valvular interstitial cell function. *Tissue engineering. Part A.* 2009;15:3221-3230.
77. Nichol JW, Koshy ST, Bae H, Hwang CM, Yamanlar S, Khademhosseini A: Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials.* 2010;31:5536-5544.
78. Chen YC, Lin RZ, Qi H, et al.: Functional Human Vascular Network Generated in Photocrosslinkable Gelatin Methacrylate Hydrogels. *Advanced functional materials.* 2012;22:2027-2039.
79. Lin RZ, Chen YC, Moreno-Luna R, Khademhosseini A, Melero-Martin JM: Transdermal regulation of vascular network bioengineering using a photopolymerizable methacrylated gelatin hydrogel. *Biomaterials.* 2013;34:6785-6796.
80. Khetan S, Burdick JA: Patterning network structure to spatially control cellular remodeling and stem cell fate within 3-dimensional hydrogels. *Biomaterials.* 2010;31:8228-8234.

81. Hanjaya-Putra D, Bose V, Shen YI, et al.: Controlled activation of morphogenesis to generate a functional human microvasculature in a synthetic matrix. *Blood.* 2011;118:804-815.
82. Hanjaya-Putra D, Wong KT, Hirotsu K, Khetan S, Burdick JA, Gerecht S: Spatial control of cell-mediated degradation to regulate vasculogenesis and angiogenesis in hyaluronan hydrogels. *Biomaterials.* 2012;33:6123-6131.
83. Kusuma S, Shen YI, Hanjaya-Putra D, Mali P, Cheng L, Gerecht S: Self-organized vascular networks from human pluripotent stem cells in a synthetic matrix. *Proceedings of the National Academy of Sciences of the United States of America.* 2013;110:12601-12606.
84. Shen YI, Cho H, Papa AE, et al.: Engineered human vascularized constructs accelerate diabetic wound healing. *Biomaterials.* 2016;102:107-119.
85. Chwalek K, Tsurkan MV, Freudenberg U, Werner C: Glycosaminoglycan-based hydrogels to modulate heterocellular communication in in vitro angiogenesis models. *Scientific reports.* 2014;4:4414.
86. Tsurkan MV, Chwalek K, Prokoph S, et al.: Defined polymer-peptide conjugates to form cell-instructive starPEG-heparin matrices in situ. *Advanced materials (Deerfield Beach, Fla.).* 2013;25:2606-2610.
87. Lutolf MP: Integration column: artificial ECM: expanding the cell biology toolbox in 3D. *Integrative biology : quantitative biosciences from nano to macro.* 2009;1:235-241.
88. Kyburz KA, Anseth KS: Synthetic mimics of the extracellular matrix: how simple is complex enough? *Ann Biomed Eng.* 2015;43:489-500.
89. Cruz-Acuna R, Garcia AJ: Synthetic hydrogels mimicking basement membrane matrices to promote cell-matrix interactions. *Matrix Biol.* 2017;57-58:324-333.
90. Guvendiren M, Burdick JA: Engineering synthetic hydrogel microenvironments to instruct stem cells. *Curr Opin Biotechnol.* 2013;24:841-846.
91. Moon JJ, Saik JE, Poche RA, et al.: Biomimetic hydrogels with pro-angiogenic properties. *Biomaterials.* 2010;31:3840-3847.
92. Cuchiara MP, Gould DJ, McHale MK, Dickinson ME, West JL: Integration of Self-Assembled Microvascular Networks with Microfabricated PEG-Based Hydrogels. *Advanced functional materials.* 2012;22:4511-4518.
93. Vigen M, Ceccarelli J, Putnam AJ: Protease-sensitive PEG hydrogels regulate vascularization in vitro and in vivo. *Macromolecular bioscience.* 2014;14:1368-1379.
94. Blache U, Metzger S, Vallmajo-Martin Q, Martin I, Djonov V, Ehrbar M: Dual Role of Mesenchymal Stem Cells Allows for Microvascularized Bone Tissue-Like Environments in PEG Hydrogels. *Adv Healthc Mater.* 2016;5:489-498.
95. Zachary I, Morgan RD: Therapeutic angiogenesis for cardiovascular disease: biological context, challenges, prospects. *Heart (British Cardiac Society).* 2011;97:181-189.
96. Wong C, Inman E, Spaethe R, Helgerson S: Fibrin-based biomaterials to deliver human growth factors. *Thrombosis and haemostasis.* 2003;89:573-582.
97. Briquez PS, Clegg LE, Martino MM, Gabhann FM, Hubbell JA: Design principles for therapeutic angiogenic materials. *Nature Reviews Materials.* 2016;1:15006.
98. Schense JC, Bloch J, Aebischer P, Hubbell JA: Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension. *Nature biotechnology.* 2000;18:415-419.
99. Schense JC, Hubbell JA: Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. *Bioconjugate chemistry.* 1999;10:75-81.

100. Ehrbar M, Djonov VG, Schnell C, et al.: Cell-demanded liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. *Circulation research.* 2004;94:1124-1132.
101. Ehrbar M, Zeisberger SM, Raeber GP, Hubbell JA, Schnell C, Zisch AH: The role of actively released fibrin-conjugated VEGF for VEGF receptor 2 gene activation and the enhancement of angiogenesis. *Biomaterials.* 2008;29:1720-1729.
102. Largo RA, Ramakrishnan VM, Marschall JS, et al.: Long-term biostability and bioactivity of "fibrin linked" VEGF121in vitro and in vivo. *Biomaterials Science.* 2014;2:581.
103. Ghajar CM, Chen X, Harris JW, et al.: The effect of matrix density on the regulation of 3-D capillary morphogenesis. *Biophysical journal.* 2008;94:1930-1941.
104. Kniazeva E, Putnam AJ: Endothelial cell traction and ECM density influence both capillary morphogenesis and maintenance in 3-D. *American journal of physiology. Cell physiology.* 2009;297:C179-187.
105. Sacchi V, Mittermayr R, Hartinger J, et al.: Long-lasting fibrin matrices ensure stable and functional angiogenesis by highly tunable, sustained delivery of recombinant VEGF164. *Proceedings of the National Academy of Sciences of the United States of America.* 2014;111:6952-6957.
106. Patterson J, Hubbell JA: Enhanced proteolytic degradation of molecularly engineered PEG hydrogels in response to MMP-1 and MMP-2. *Biomaterials.* 2010;31:7836-7845.
107. Patterson J, Hubbell JA: SPARC-derived protease substrates to enhance the plasmin sensitivity of molecularly engineered PEG hydrogels. *Biomaterials.* 2011;32:1301-1310.
108. Zisch AH, Lutolf MP, Ehrbar M, et al.: Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2003;17:2260-2262.
109. Phelps EA, Landazuri N, Thule PM, Taylor WR, Garcia AJ: Bioartificial matrices for therapeutic vascularization. *Proceedings of the National Academy of Sciences of the United States of America.* 2010;107:3323-3328.
110. Martino MM, Hubbell JA: The 12th-14th type III repeats of fibronectin function as a highly promiscuous growth factor-binding domain. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2010;24:4711-4721.
111. Martino MM, Briquez PS, Ranga A, Lutolf MP, Hubbell JA: Heparin-binding domain of fibrin(ogen) binds growth factors and promotes tissue repair when incorporated within a synthetic matrix. *Proceedings of the National Academy of Sciences of the United States of America.* 2013;110:4563-4568.
112. Zieris A, Prokoph S, Levental KR, et al.: FGF-2 and VEGF functionalization of starPEG-heparin hydrogels to modulate biomolecular and physical cues of angiogenesis. *Biomaterials.* 2010;31:7985-7994.
113. Martino MM, Briquez PS, Guc E, et al.: Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing. *Science (New York, N.Y.).* 2014;343:885-888.
114. Wylie RG, Ahsan S, Aizawa Y, Maxwell KL, Morshead CM, Shoichet MS: Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nature materials.* 2011;10:799-806.

115. Metzger S, Lienemann PS, Ghayor C, et al.: Modular poly(ethylene glycol) matrices for the controlled 3D-localized osteogenic differentiation of mesenchymal stem cells. *Advanced healthcare materials.* 2015;4:550-558.
116. Levenberg S, Huang NF, Lavik E, Rogers AB, Itskovitz-Eldor J, Langer R: Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100:12741-12746.
117. Levenberg S, Rouwkema J, Macdonald M, et al.: Engineering vascularized skeletal muscle tissue. *Nature biotechnology.* 2005;23:879-884.
118. Liu CH, Wang Z, Sun Y, Chen J: Animal models of ocular angiogenesis: from development to pathologies. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology.* 2017.
119. Walchli T, Wacker A, Frei K, et al.: Wiring the Vascular Network with Neural Cues: A CNS Perspective. *Neuron.* 2015;87:271-296.
120. Scott AW, Bressler SB: Long-term follow-up of vascular endothelial growth factor inhibitor therapy for neovascular age-related macular degeneration. *Current opinion in ophthalmology.* 2013;24:190-196.
121. Jain RK: Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. *Cancer cell.* 2014;26:605-622.
122. Simons M, Alitalo K, Annex BH, et al.: State-of-the-Art Methods for Evaluation of Angiogenesis and Tissue Vascularization: A Scientific Statement From the American Heart Association. *Circulation research.* 2015;116:e99-132.
123. Lienemann PS, Devaud YR, Reuten R, et al.: Locally controlling mesenchymal stem cell morphogenesis by 3D PDGF-BB gradients towards the establishment of an in vitro perivascular niche. *Integrative biology : quantitative biosciences from nano to macro.* 2015;7:101-111.
124. Zhang J, Schwartz MP, Hou Z, et al.: A Genome-wide Analysis of Human Pluripotent Stem Cell-Derived Endothelial Cells in 2D or 3D Culture. *Stem Cell Reports.* 2017;8:907-918.
125. Muller K, Naumann S, Weber W, Zurbriggen MD: Optogenetics for gene expression in mammalian cells. *Biological chemistry.* 2015;396:145-152.
126. Brown TE, Anseth KS: Spatiotemporal hydrogel biomaterials for regenerative medicine. *Chemical Society reviews.* 2017.
127. Bergers G, Hanahan D: Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer.* 2008;8:592-603.
128. Jain RK: Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2013;31:2205-2218.
129. Song HH, Park KM, Gerecht S: Hydrogels to model 3D in vitro microenvironment of tumor vascularization. *Advanced drug delivery reviews.* 2014;79-80:19-29.
130. Chwalek K, Bray LJ, Werner C: Tissue-engineered 3D tumor angiogenesis models: potential technologies for anti-cancer drug discovery. *Advanced drug delivery reviews.* 2014;79-80:30-39.
131. Taubenberger AV, Bray LJ, Haller B, et al.: 3D extracellular matrix interactions modulate tumour cell growth, invasion and angiogenesis in engineered tumour microenvironments. *Acta biomaterialia.* 2016;36:73-85.
132. Bray LJ, Binner M, Holzheu A, et al.: Multi-parametric hydrogels support 3D in vitro bioengineered microenvironment models of tumour angiogenesis. *Biomaterials.* 2015;53:609-620.

133. Roudsari LC, Jeffs SE, Witt AS, Gill BJ, West JL: A 3D Poly(ethylene glycol)-based Tumor Angiogenesis Model to Study the Influence of Vascular Cells on Lung Tumor Cell Behavior. *Scientific reports.* 2016;6:32726.
134. Bertassoni LE, Cecconi M, Manoharan V, et al.: Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab Chip.* 2014;14:2202-2211.
135. Kageyama T, Kakegawa T, Osaki T, et al.: Rapid engineering of endothelial cell-lined vascular-like structures in in situ crosslinkable hydrogels. *Biofabrication.* 2014;6:025006.
136. Arrigoni C, Bongio M, Talo G, et al.: Rational Design of Prevascularized Large 3D Tissue Constructs Using Computational Simulations and Biofabrication of Geometrically Controlled Microvessels. *Advanced healthcare materials.* 2016;5:1617-1626.
137. Zheng Y, Chen J, Craven M, et al.: In vitro microvessels for the study of angiogenesis and thrombosis. *Proceedings of the National Academy of Sciences of the United States of America.* 2012;109:9342-9347.
138. Nguyen DH, Stapleton SC, Yang MT, et al.: Biomimetic model to reconstitute angiogenic sprouting morphogenesis in vitro. *Proceedings of the National Academy of Sciences of the United States of America.* 2013;110:6712-6717.
139. Kolesky DB, Homan KA, Skylar-Scott MA, Lewis JA: Three-dimensional bioprinting of thick vascularized tissues. *Proceedings of the National Academy of Sciences of the United States of America.* 2016;113:3179-3184.
140. Kolesky DB, Truby RL, Gladman AS, Busbee TA, Homan KA, Lewis JA: 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Advanced materials (Deerfield Beach, Fla.).* 2014;26:3124-3130.
141. Lee VK, Kim DY, Ngo H, et al.: Creating perfused functional vascular channels using 3D bio-printing technology. *Biomaterials.* 2014;35:8092-8102.
142. Brandenberg N, Lutolf MP: In Situ Patterning of Microfluidic Networks in 3D Cell-Laden Hydrogels. *Advanced materials (Deerfield Beach, Fla.).* 2016;28:7450-7456.
143. Wang X, Phan DT, Sobrino A, George SC, Hughes CC, Lee AP: Engineering anastomosis between living capillary networks and endothelial cell-lined microfluidic channels. *Lab Chip.* 2016;16:282-290.
144. Wang X, Phan DTT, Zhao D, George SC, Hughes CCW, Lee AP: An on-chip microfluidic pressure regulator that facilitates reproducible loading of cells and hydrogels into microphysiological system platforms. *Lab Chip.* 2016;16:868-876.
145. Hsu YH, Moya ML, Hughes CC, George SC, Lee AP: A microfluidic platform for generating large-scale nearly identical human microphysiological vascularized tissue arrays. *Lab Chip.* 2013;13:2990-2998.
146. Yeon JH, Ryu HR, Chung M, Hu QP, Jeon NL: In vitro formation and characterization of a perfusable three-dimensional tubular capillary network in microfluidic devices. *Lab Chip.* 2012;12:2815-2822.
147. van Duinen V, Trietsch SJ, Joore J, Vulto P, Hankemeier T: Microfluidic 3D cell culture: from tools to tissue models. *Curr Opin Biotechnol.* 2015;35:118-126.
148. Phan DTT, Wang X, Craver BM, et al.: A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. *Lab Chip.* 2017;17:511-520.
149. Sobrino A, Phan DT, Datta R, et al.: 3D microtumors in vitro supported by perfused vascular networks. *Scientific reports.* 2016;6:31589.
150. Nguyen EH, Daly WT, Le NNT, et al.: Versatile synthetic alternatives to Matrigel for vascular toxicity screening and stem cell expansion. 2017;1:0096.

Figure legends:

Figure 1: Engineering neovascularization in hydrogels inspired by the template function of the extracellular matrix (ECM) during blood vessel development and maturation. A) The establishment of new blood capillaries occurs through vasculogenesis and angiogenesis in an angio-competent milieu generated by growth factors and the ECM. B) Tools for vascular engineering derived from the natural processes of blood vessel development include vascular cells, growth factors, and ECM-inspired hydrogel scaffolds. Hydrogels can be engineered from natural ECM components or synthetic ECM analogues. C) Hydrogel-based strategies to generate new functional vascular networks in vivo. Pre-vascularization follows the concept of engineering vascular networks within hydrogels in vitro by the application of endothelial cells that self-assemble into micro-capillary networks. Upon transplantation, pre-vascularized hydrogel constructs can anastomose to the host vasculature and become perfused. In an alternative strategy, the delivery of soluble, matrix bound, or on demand releasable angiogenic growth factors can promote the formation of new vessels in situ.

Figure 2: Pre-vascularization of fibrin hydrogels leads to the rapid anastomosis of engineered micro-capillaries with the host vasculature. Micro-capillaries were engineered in vitro by the co-culture of human fibroblasts with human umbilical vein endothelial cells (HUVEC) in fibrin hydrogels. Pre-vascularized tissue (A, 7 days in vitro pre-culture) was perfused by host blood cells more rapidly than non-pre-vascularized tissue (B, 1 day in vitro pre-culture) upon implantation. C) HUVEC-cultures in the absence of supporting fibroblasts do not form perfusable structures. Histological tissue sections from 3–14 days post-implantation, with red blood cells being evident in the pre-vascularized tissue starting at day 5 post-implantation. MT, mouse tissue; IT, implant tissue. Scale bars: 100 mm. Adapted figure from Chen et al.[56] with re-print permission.

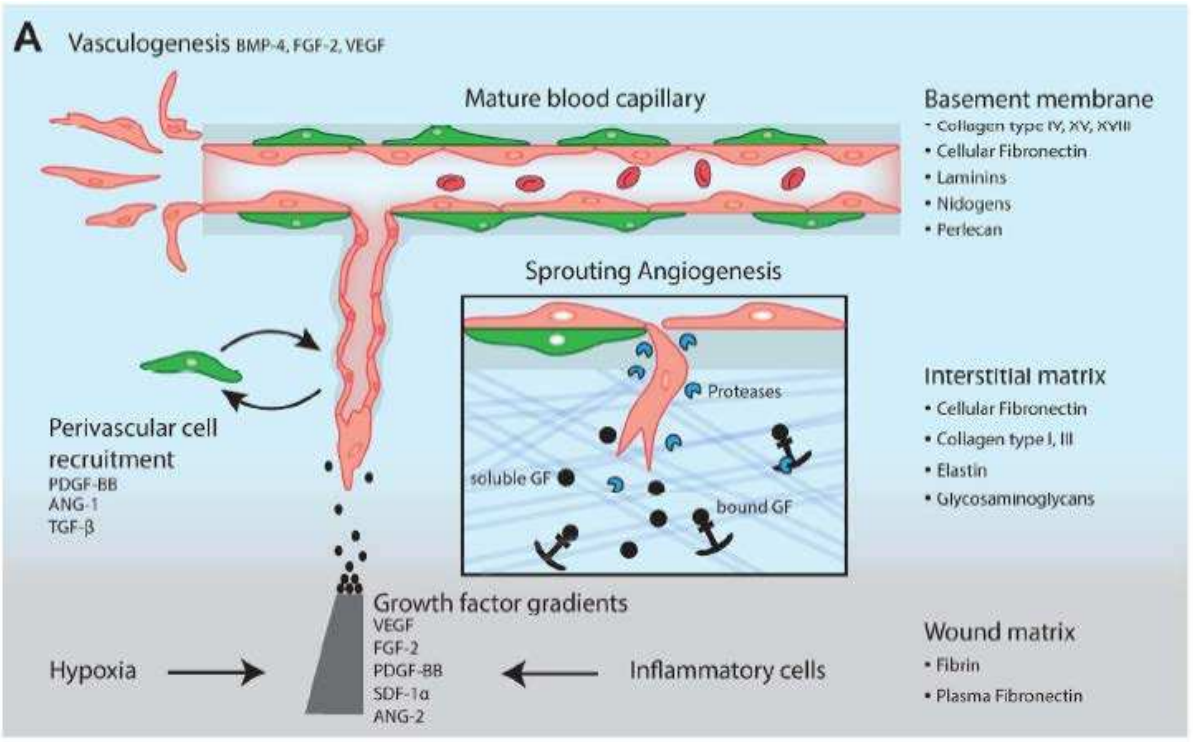
Figure 3: In situ neovascularization by engineered, hydrogel-immobilized VEGF delivery. A) Skin vascularization response to fibrin hydrogels containing no VEGF (control), soluble VEGF, or engineered, fibrin-immobilized VEGF. Although the immobilized VEGF outperforms soluble VEGF, the vascularization response decreases as the fibrin hydrogel becomes degraded over time. Figure re-print from Largo et al.[102] B) Improvement of fibrin-immobilized VEGF treatment by aprotinin engineered, long-lasting fibrin hydrogels. No VEGF (control) or engineered, fibrin-immobilized VEGF (0.5 μg/mL and 5 μg/mL) were compared in a hindlimb ischemia mouse model. Fibrin hydrogels used for the delivery were stabilized by fibrin-immobilization of the fibrinolysis inhibitor aprotinin. Tissues analyzed 4 weeks after hydrogel delivery for endothelial cells (CD31, in red), pericytes (NG2, in green), and smooth-muscle cells (α-SMA, in cyan, scale bar: 20 μm) or for microcirculation by Laser-Doppler-Imaging of non-ischemic and ischemic limbs (left and right legs, respectively). Figure re-print from Sacchi et al.[105]

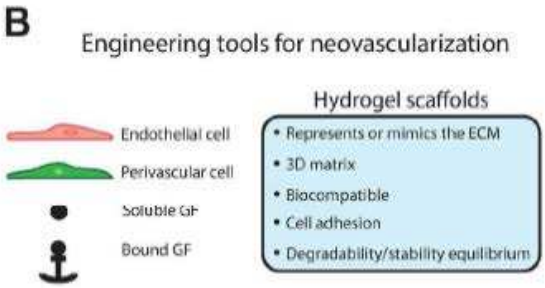
Figure 4: Perfused vasculature on-a-chip model as potential cancer and drug screening platform. A) Vascular networks formed by co-culture of human endothelial colony forming cells (ECFC-EC) and human fibroblasts in fibrin hydrogels. Vascular networks inside the three tissue chambers connect to microfluidic channels and can be perfused (70 kDa FITC-dextran). B) Vascularized microtumors (VMTs). HCT116 cancer cells were embedded together with endothelial/fibroblast co-cultures into fibrin hydrogels and grow into microtumors surrounded by perfused vascular networks. Scale bars: 100 μm. C) VMTs used for anti-cancer drug screening. The collapse of the tumor (vasculature) can be monitored upon treatment with FDA-approved anti-cancer drugs. Adapted figure from Phan et al.[148] with re-print permission.

Figure 5: Engineering of vascularized tissue models using hydrogels.

Vascularized tissue models largely depend on engineered hydrogel-based, cell-instructive microenvironments. The culture of adult (stem) cells in these cell-instructive microenvironments enables the study of basic vascular biology under defined in vitro conditions. Engineering physiologically relevant human tissue and cancer models requires the integration of knowhow from vascular biology and biofabrication techniques. In the future, the use of defined 3D culture systems together with patient derived cells will allow for the screening of therapeutics and the testing for treatment towards personalized medicine.

Figure 1





Features	Natural ECM	(Semi) Synthetic ECM
Cell adhesion	Inherent	Requires engineering
Degradability	Inherent	Requires engineering
Source	Animal tissue	Chemically defined
Advantage	Well-known native bioactivity	Modular design Design freedom
Disadvantage	Low mechanical and proteolytical stability	Requires engineering
Materials	Collagen I, Fibrin, Gelatin	HA, Heparin-PEG, PEG

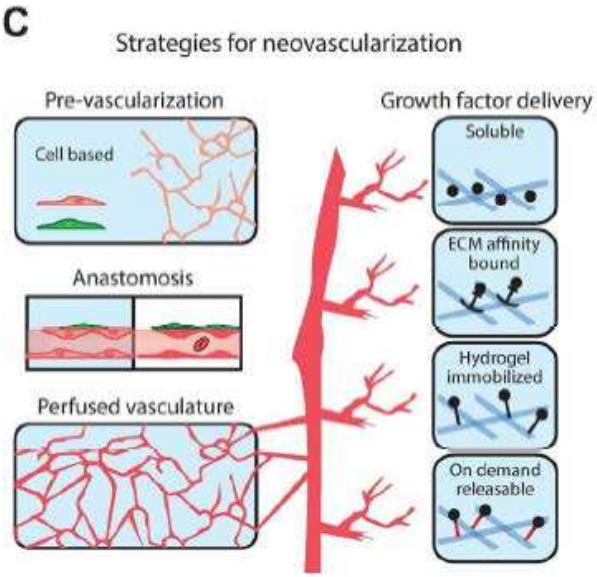


Figure 2

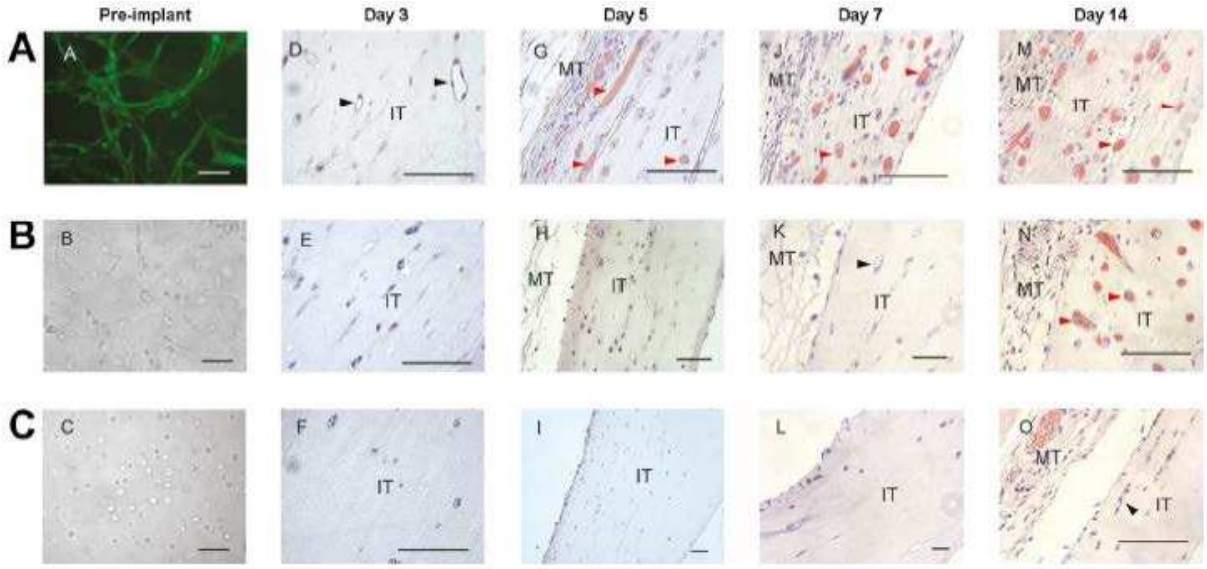


Figure 3

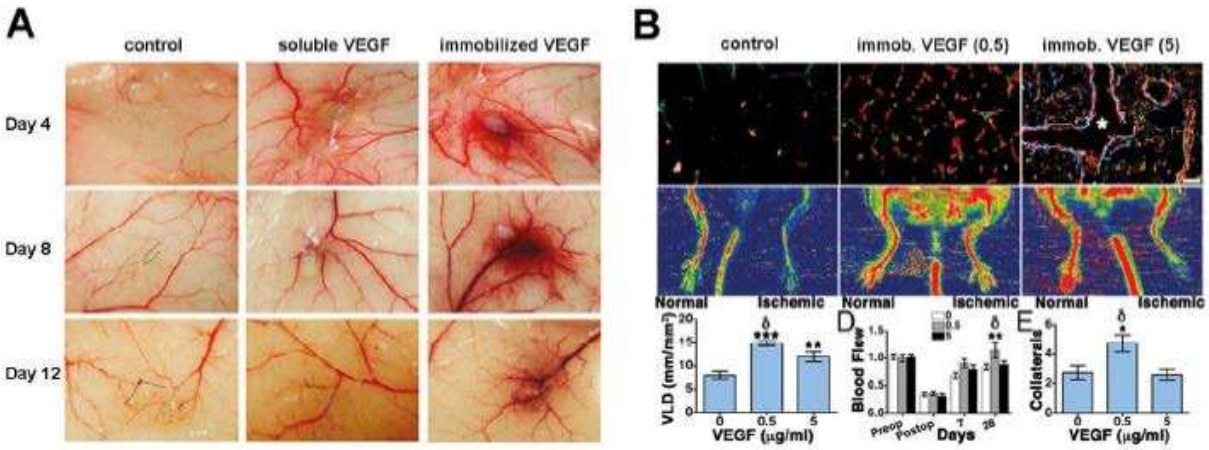


Figure 4

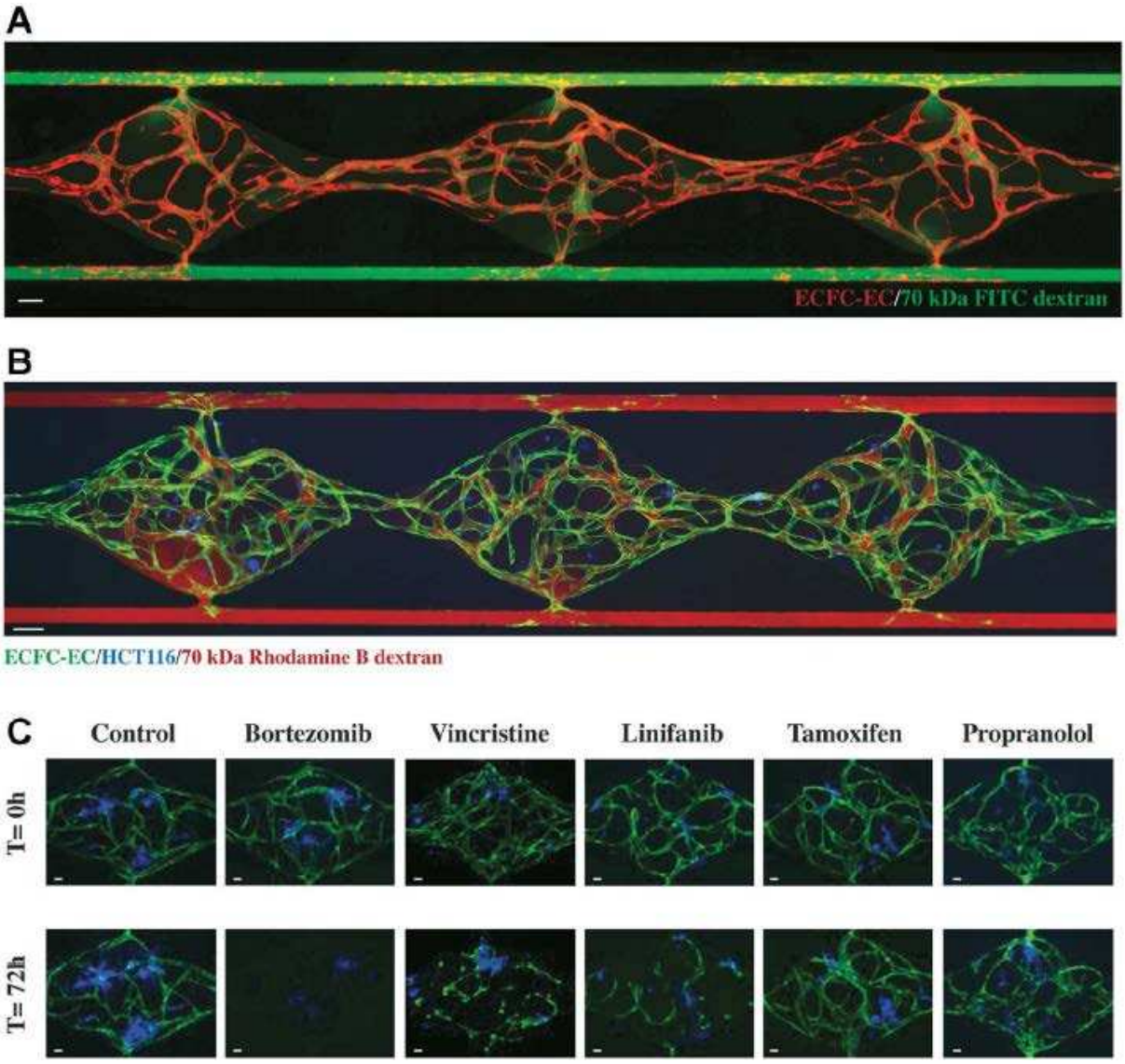
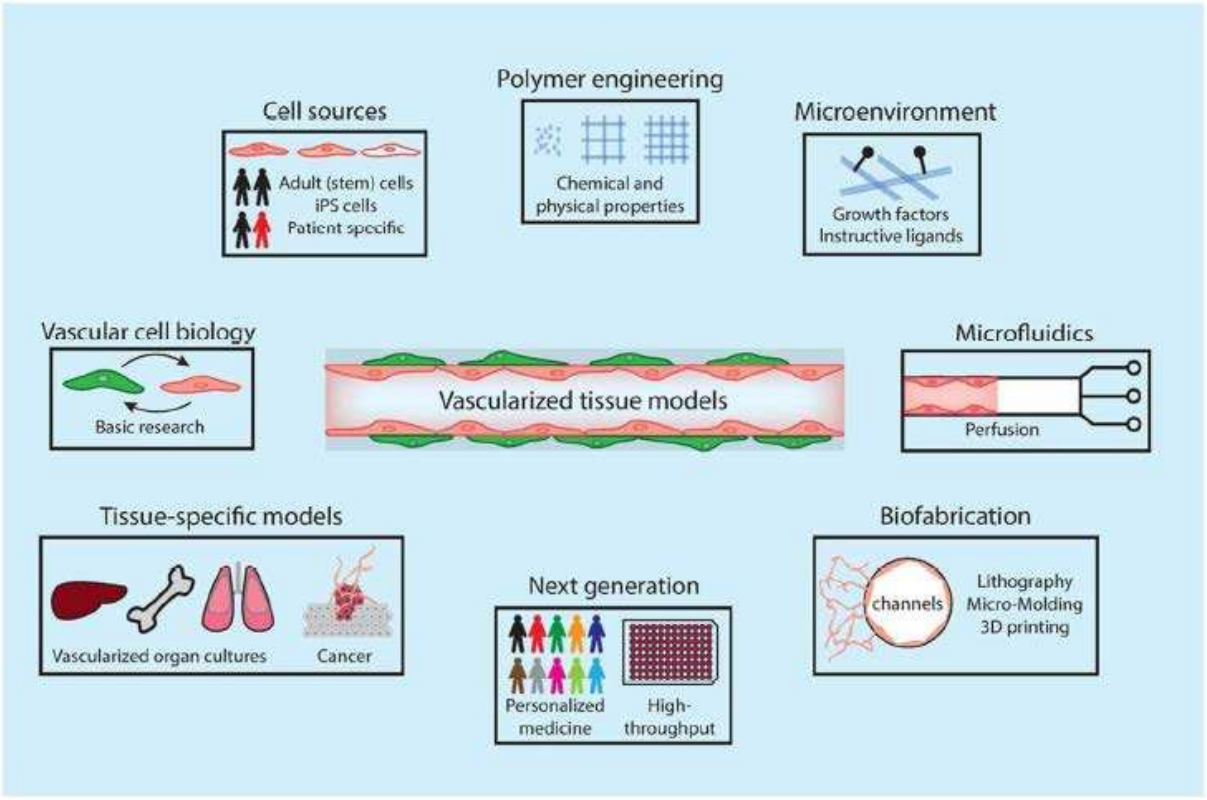

A
ECFC-EC/70 kDa FITC dextran
B
ECFC-EC/HCT116/70 kDa Rhodamine B dextran
C
Control
Bortezomib
Vincristine
Linifanib
Tamoxifen
Propranolol
T= 0h
T= 72h

Figure 5


Cell sources
Adult (stem) cells
iPS cells
Patient specific
Polymer engineering
Chemical and
physical properties
Microenvironment
Growth factors
Instructive ligands
Vascular cell biology
Basic research
Vascularized tissue models
Microfluidics
Perfusion
Tissue-specific models
Vascularized organ cultures
Cancer
Next generation
Personalized
medicine
High-
throughput
Biofabrication
channels
Lithography
Micro-Molding
3D printing